Lipoprotein-Associated Phospholipase A₂ Adds to Risk Prediction of Incident Coronary Events by C-Reactive Protein in Apparently Healthy Middle-Aged Men From the General Population

Results From the 14-Year Follow-Up of a Large Cohort From Southern Germany

Wolfgang Koenig, MD; Natalie Khuseyinova, MD; Hannelore Löwel, MD; Gerlinde Trischler; Christa Meisinger, MD, MPH

Background—Chronic inflammation represents an essential feature of the atherosclerotic process. Lipoprotein-associated phospholipase A₂ (Lp-PLA₂), an enzyme mainly produced by monocytes/macrophages, generates potent proinflammatory products.

Methods and Results—Plasma concentrations of Lp-PLA₂ were determined by ELISA in 934 apparently healthy men aged 45 to 64 years sampled from the general population in 1984 and followed up until 1998. During this period, 97 men experienced a coronary event diagnosed according to the MONICA (MONItoring of trends and determinants in CArdiovascular disease) protocol. Baseline levels of Lp-PLA₂ were higher in subjects who experienced an event than in event-free subjects (295±113 versus 263±79 ng/mL, P<0.01). Lp-PLA₂ was positively correlated with total cholesterol (R=0.30, P<0.0001) and age (R=0.12, P=0.001), was only slightly correlated with HDL cholesterol (R=0.09, P=0.005) and C-reactive protein (R=0.06, P=0.06), but was not correlated with body mass index or blood pressure. In a Cox model, a 1-SD increase in Lp-PLA₂ was associated with risk of future coronary events (hazard ratio [HR] 1.37, 95% CI 1.16 to 1.62). After controlling for potential confounders, the HR was attenuated but remained statistically significant (HR 1.21, 95% CI 1.01 to 1.45).

Conclusions—Elevated levels of Lp-PLA₂ appeared to be predictive of future coronary events in apparently healthy middle-aged men with moderately elevated total cholesterol, independent of CRP. This suggests that Lp-PLA₂ and CRP may be additive in their ability to predict risk of coronary heart disease. (Circulation. 2004;110:1903-1908.)

Key Words: inflammation ▪ risk factors ▪ coronary disease ▪ epidemiology ▪ prognosis

Although most patients with coronary heart disease (CHD) do have at least 1 major risk factor, the same can be said for most middle-aged and elderly subjects without CHD.¹ ² Thus, classic risk factors cannot account for all incident coronary events, and there may be a substantial number of subjects without elevated lipoprotein concentrations who have the disease.³ ⁴ Even following current recommendations to use global risk assessment for risk stratification in asymptomatic subjects leaves a large proportion of the population at intermediate risk,⁵ where no established strategies are available. Such an obvious gap has fueled the search for additional diagnostic tools. Because inflammation plays an important role in atherogenesis and its clinical complications, in recent years, a large number of biomarkers of inflammation have been investigated in prospective studies and have been found to be associated with cardiovascular events.⁶ For a number of reasons, the most promising marker at present seems to be C-reactive protein (CRP).⁷ Indeed, recently, it could be shown that adding CRP to the Framingham risk score resulted in improved risk prediction, in particular in those in the intermediate range.⁸ However, increasing evidence suggests that a variety of inflammatory molecules are involved in the atherothrombotic process,⁹ and not all of them may reflect the same pathophysiology, thus raising the interesting question of whether or not the combination of different markers may further improve risk prediction.
Lipoprotein-associated phospholipase A₂ (Lp-PLA₂) is an enzyme that may directly promote atherogenesis by generating potent proinflammatory and prothrombotic products, such as lysophosphatidylcholine and oxidized free fatty acids from oxidation of LDL. An important step in atherogenesis, Lp-PLA₂ is produced mainly by the characteristic cells of the atherosclerotic plaque, namely, monocytes/macrophages, T lymphocytes, and mast cells. Furthermore, Lp-PLA₂ has been detected in both human and rabbit atherosclerotic lesions. Experimental studies in Watanabe heritable hyperlipidemic rabbits have demonstrated that inhibition of Lp-PLA₂ leads to the reduction of atherosclerotic lesion formation. In the bloodstream, two thirds of the Lp-PLA₂ plasma isoform circulates primarily bound to LDL; the other third is distributed between HDL and VLDL.

We sought to investigate simultaneously the association between plasma concentrations of Lp-PLA₂, CRP, and long-term risk of CHD in initially healthy middle-aged men from the general population in Augsburg, Southern Germany.

Methods

Study Design, Population, and Follow-Up

The population-based MONICA (MONItoring of trends and determinants in Cardiovascular disease) Augsburg survey, conducted in 1984/1985, was used as the database. The MONICA Augsburg project was part of the multinational World Health Organization MONICA project. Briefly, 4022 of the 5069 eligible individuals aged 25 to 64 years initially sampled at random from a study population of 282,279 inhabitants of a mixed urban/rural area participated in the first survey (response rate 79.3%). The present report is based on all 1074 men aged 45 to 64 years. Of these subjects, 140 men were not included in the analyses because of a previous myocardial infarction (MI), missing values for CRP or Lp-PLA₂, or control variables. Thus, a total of 934 men aged 45 to 64 years had data on all variables studied.

In a follow-up study in 1998, the vital status was assessed for all sampled persons of the first MONICA survey in 1984. During the 14-year observation period, 209 men aged 45 to 64 years at baseline had died, and vital status could not be assessed for 1 man. The outcome variable was a combination of incident fatal or nonfatal acute MI and sudden cardiac death. They were identified through the MONICA/KORA (Kooperative Gesundheitsforschung in der Region Augsburg) coronary event registry of the 25- to 74-year-old study population and censored at the 75th year of age. According to the MONICA manual, the diagnosis of a major nonfatal MI event was based on symptoms, cardiac enzymes, and typical ECG changes. Deaths from cardiovascular causes were validated by autopsy reports, death certificates, chart review, and information from the last treating physician.

Survey Methods

All participants completed a standardized questionnaire, including medical history, lifestyle, and drug use history. Blood pressure, body height (m) and body weight (kg), body mass index (BMI, m/kg²), smoking behavior, and alcohol consumption (g/d) were determined as described elsewhere. Leisure-time physical activity was assessed on a 4-level graded scale for winter and summer. The number of education years was calculated on the basis of the highest level of formal education completed.

Laboratory Procedures

A nonfasting venous blood sample was collected from all participants in a supine resting position. Samples for measurement of CRP and Lp-PLA₂ were stored at −70°C until analysis. Serum CRP concentrations were measured with a high-sensitivity (hs) immunoradiometric assay (range 0.05 to 10 mg/L) as described previously. The coefficient of variation for repeated measurements was 12% over all ranges. Plasma levels of Lp-PLA₂ were determined with a commercial Lp-PLA₂-ELISA kit (PLAC test; supplied by diaDexus Inc, South San Francisco, Calif). The lower detection limit of Lp-PLA₂ in this assay is ~2 ng/mL. The intercept coefficient of variation was 9.6%. Total serum cholesterol (TC) and HDL cholesterol (HDL-C) were measured by routine enzymatic methods. Corresponding coefficients of variation were between 1 and 3% for TC, and between 3 and 4% for HDL-C. All analyses were run in a blinded fashion.

Statistical Analysis

Means or proportions for baseline demographic and clinical characteristics were computed for men with and without an incident coronary event. The distribution of CRP concentrations and the TC/HDL-C ratio were markedly skewed and were therefore log-transformed. Differences between continuous variables were tested for statistical significance by means of t tests, and proportions were tested by χ² test. We assessed associations among variables with use of Pearson’s correlation coefficient. Cox proportional hazards analysis was used to assess the independent risk for the occurrence of a first coronary event for an increase of 1 SD of CRP and Lp-PLA₂. Results are presented as hazard ratios (HRs) together with their 95% CIs. First, crude HRs were calculated. Results were then adjusted for age (continuous), regular smoking (yes/no), and history of diabetes (yes/no) and were further adjusted for the TC/HDL-C ratio (continuous), BMI (continuous), systolic blood pressure (continuous), alcohol consumption (0 g/d, 0.1 to 39.9 g/d, ≥40 g/d), physical activity (inactive/active), and education years (<12 or ≥12 years) in all other models. Finally, the potential additive effect of CRP and Lp-PLA₂ was evaluated. For this purpose, high CRP was defined as >3.0 mg/L, and high Lp-PLA₂ was defined as ≥290.8 ng/mL (upper tertile cutpoint). A similar stepwise analytical procedure was chosen. Probability values are based on the Wald statistic. Significance tests are 2 tailed, and probability values <0.05 were considered statistically significant. All analyses were performed with the Statistical Analysis System (version 8.2, SAS Institute Inc).

Results

Baseline Characteristics

During an average follow-up of 14 years, a total of 97 fatal and nonfatal coronary events, including sudden cardiac death, occurred. Men with an acute event were significantly older, had higher TC and HDL-C, and had a higher TC/HDL-C ratio. They were more frequently smokers, were less frequently physically active, and had a higher prevalence of diabetes. Concentrations of both Lp-PLA₂ and CRP were considerably higher than in those free of an event during follow-up (Table 1).

Correlation Between Lp-PLA₂, CRP, and Other Risk Factors

Lp-PLA₂ was positively correlated with age (R=0.12, P=0.0001) and TC (R=0.30, P<0.0001). It was only slightly correlated with HDL-C (R=0.09, P=0.005) but was not correlated with smoking, BMI, or systolic blood pressure. The latter, however, were correlated with CRP (Table 2). Lp-PLA₂ concentration was only marginally correlated with CRP (R=0.06, P=0.06).

Lp-PLA₂, CRP, and Risk of a First Coronary Event

In a model containing only Lp-PLA₂, a 1-SD increase in the marker was associated with a 37% increase in coronary risk in unadjusted analyses (HR 1.37, 95% CI 1.16 to 1.62;
TABLE 2. Correlation Between Lp-PLA2, CRP, and Other Cardiovascular Risk Factors in the MONICA/KORA Cohort Study 1984–1998 (n=934)

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Lp-PLA2</th>
<th>CRP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.12 (0.0001)</td>
<td>0.15 (-0.0001)</td>
</tr>
<tr>
<td>TC</td>
<td>0.30 (+0.0001)</td>
<td>0.07 (0.03)</td>
</tr>
<tr>
<td>HDL-C</td>
<td>0.09 (0.005)</td>
<td>-0.12 (0.0002)</td>
</tr>
<tr>
<td>TCHDL-C</td>
<td>0.07 (0.04)</td>
<td>0.13 (-0.0001)</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>0.02 (0.50)</td>
<td>0.11 (0.0007)</td>
</tr>
<tr>
<td>BMI</td>
<td>-0.06 (0.05)</td>
<td>0.21 (-0.0001)</td>
</tr>
<tr>
<td>CRP</td>
<td>0.06 (0.06)</td>
<td>1.00</td>
</tr>
</tbody>
</table>

"*Log transformed."

TABLE 3. HR for CHD by a 1-SD Increase in Lp-PLA2 or CRP, Each in a Separate Model, or Combined in the Same Model: MONICA/KORA Cohort Study 1984–1998 (n=934)

<table>
<thead>
<tr>
<th>Model</th>
<th>HR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lp-PLA2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted</td>
<td>1.37 (1.16–1.62)</td>
<td>0.0002</td>
</tr>
<tr>
<td>Adjusted for age, DM, smoking</td>
<td>1.26 (1.05–1.54)</td>
<td>0.01</td>
</tr>
<tr>
<td>Multivariable adjustment*</td>
<td>1.23 (1.02–1.47)</td>
<td>0.03</td>
</tr>
<tr>
<td>CRP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted</td>
<td>1.57 (1.30–1.91)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Adjusted for age, DM, smoking</td>
<td>1.32 (1.07–1.62)</td>
<td>&lt;0.009</td>
</tr>
<tr>
<td>Multivariable adjustment*</td>
<td>1.28 (1.03–1.60)</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td>Lp-PLA2 and CRP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted</td>
<td>1.35 (1.14–1.60)</td>
<td>0.0006</td>
</tr>
<tr>
<td>CRP</td>
<td>1.55 (1.28–1.89)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Adjusted for age, DM, smoking</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lp-PLA2</td>
<td>1.25 (1.04–1.50)</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>CRP</td>
<td>1.31 (1.07–1.62)</td>
<td>0.01</td>
</tr>
<tr>
<td>Multivariable adjustment*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lp-PLA2</td>
<td>1.21 (1.01–1.45)</td>
<td>0.04</td>
</tr>
<tr>
<td>CRP</td>
<td>1.27 (1.01–1.59)</td>
<td>0.04</td>
</tr>
</tbody>
</table>

BP indicates blood pressure. Values are percentages or mean (SD), unless otherwise indicated. For the comparison between men with MI and men without MI, t test was used for continuous variables, and χ² test was used for categorical variables. *P<0.05, †P<0.01, ‡P<0.001.

Values are geometric mean (SD) calculated from log-transformed distribution. P=0.0002. Multivariable adjustment for traditional risk factors reduced the HR to 1.23 (95% CI 1.02 to 1.47), which was still statistically significant (Table 3). Similar analyses for CRP resulted in an HR of 1.57 (95% CI 1.30 to 1.91) in unadjusted analyses, which was reduced to 1.28 (95% CI 1.03 to 1.60) after multivariable adjustments had been made (Table 3).

Combined Analysis of Inflammatory Markers and Risk of a First Coronary Event

When both markers were simultaneously assessed in the same model, they still independently predicted future coronary events in unadjusted analyses, with only slightly reduced HRs for Lp-PLA2 (1.35, 95% CI 1.14 to 1.60) and CRP (1.55, 95% CI 1.28 to 1.89). This held true in further analyses that took into account a variety of traditional risk factors, in which the decrease of the HR for CRP was somewhat stronger (1.27, 95% CI 1.01 to 1.59) than for the HR for Lp-PLA2 (1.21, 95% CI 1.01 to 1.45; Table 3).

Finally, we looked at the potential additive effect of both markers for risk prediction. For this purpose, we defined high CRP according to a recent American Heart Association/Centers for Disease Control and Prevention consensus document as >3.0 mg/L. For Lp-PLA2, the upper tertile cutpoint (290.8 ng/mL) was used. Again, a similar stepwise analytical procedure was chosen, with examination first of the unadjusted relationship, then with adjustment for age, diabetes, and smoking, and finally, with additional adjustment for the remainder of the potential confounders. The Figure shows that the combination of both elevated Lp-PLA2 and elevated CRP was consistently associated with a statistically significantly increased risk for future coronary events and was superior to either elevated marker alone in predicting risk, with an HR of 1.93 (95% CI 1.09 to 3.40) compared with both markers not being increased (referent) in the fully adjusted model.

Discussion

In this prospective cohort study, we directly compared the relative risk for future coronary events associated with elevated Lp-PLA2 and with elevated CRP concentrations.
Based on the results from the Women’s Heart Health Study (WHS), a low-risk population for cardiovascular diseases, baseline concentrations of Lp-PLA2 were associated with an 18% increase in risk for cardiovascular disease when followed up for 5 years. In that study, a 1-SD increase in Lp-PLA2 was associated with an 18% increase in risk in multivariable adjustments. Finally, the present results show an additive effect of both markers on risk prediction.

Prospective Evidence for the Association Between Lp-PLA2 and CHD From Other Studies

To date, 3 prospective studies have reported on the association between elevated Lp-PLA2 and future cardiovascular risk in different populations. Initial evidence for such an association came from the West of Scotland Coronary Prevention Study (WOSCOPS) in hyperlipidemic, middle-aged men followed up for 5 years. In that study, a 1-SD increase in Lp-PLA2 was associated with an 18% increase in risk in multivariable analysis, even after controlling for other markers of inflammation, compared with a 21% increased risk for coronary events in the present study. These results are very similar despite the fact that the 2 study populations were clearly different in both their TC level, which was 272 mg/dL in WOSCOPS and 245 mg/dL in MONICA/KORA, and their HDL-C level, which was 44 and 51.3 mg/dL, respectively. Other characteristics were comparable, except for BMI, which was slightly higher in Augsburg. In addition, we studied a full cohort sampled from the general population with a much longer follow-up of 14 years compared with a case-cohort study with a follow-up of 5 years within WOSCOPS, a primary prevention trial to evaluate the effect of pravastatin on coronary events. Thus, the present data show for the first time that Lp-PLA2 is able to predict risk in the long term.

The predictive role of Lp-PLA2 has been assessed in the Women’s Health Study (WHS), a low-risk population for cardiovascular diseases. Baseline concentrations of Lp-PLA2 were increased among women who subsequently developed cardiovascular events (including 74 cases of MI and 49 with stroke) compared with those who remained free of vascular disease (mean 1.20 versus 1.05 mg/L, respectively; P=0.016). However, the relative risk in the top quartile compared with the bottom quartile was 1.73 (95% CI 0.87 to 3.44) and thus statistically nonsignificant and decreased further to 1.17 (95% CI 0.45 to 3.05) after adjustment for various risk factors. This lack of association is in contrast to findings from WOSCOPS and the present cohort study and may be attributable to gender differences of Lp-PLA2, because only women participated in WHS compared with WOSCOPS and the present study in men only. Indeed, several studies have already reported lower concentrations of Lp-PLA2 in women than in men.

The third prospective study has recently been published based on data from the Atherosclerosis Risk in Communities (ARIC) study. A case-cohort design was chosen, and 608 men and women with incident CHD were compared with 740 controls from a cohort random sample and followed up for 6 to 8 years. Again, Lp-PLA2 concentrations were higher in cases than in controls. In age- and gender-adjusted analysis, Lp-PLA2 was associated with increased risk (HR in the top tertile compared with the bottom tertile was 1.78; 95% CI 1.33 to 2.38), but statistical significance was lost after multivariable adjustments. In contrast, CRP was predictive in overall analysis even after multivariable adjustments (HR 1.72; 95% CI 1.24 to 2.39 comparing those with CRP >3.0 mg/L with those with CRP <1.0 mg/L). However, the authors found a significant interaction between Lp-PLA2 and LDL cholesterol (<130 and ≥130 mg/dL). In subjects with low LDL cholesterol, Lp-PLA2 was significantly and independently predicted CHD (HR 2.08, 95% CI 1.20 to 3.62). Individuals with high Lp-PLA2 (≥422 μg/L) and high CRP (>3 mg/L) in this subgroup exhibited the largest risk for CHD (HR 2.95, 95% CI 1.47 to 5.94). Compared with the present study, ARIC had included females, blacks, and more subjects with diabetes; participants also had a higher
BMI but lower blood pressures and TC and similar HDL-C values. Despite these differences, Lp-PLA₂ and CRP were found to be complementary predictors for individuals at high risk in both studies, although in ARIC, this was true only in those with low LDL cholesterol.

Correlations Between Lp-PLA₂ and Other Risk Factors
In all 4 studies, a strong correlation was seen between Lp-PLA₂ and either TC or LDL cholesterol. Discrepant findings have been reported for HDL-C, with inverse correlations in WHS and ARIC and small positive correlations in WOSCOPS and MONICA/KORA. These differences cannot be explained so far. Interestingly, in all studies, correlations with other traditional risk factors were negligible, and in particular, no relevant correlation was seen with CRP. In contrast, CRP has been shown to correlate with most traditional risk factors, the most pronounced correlations being seen with smoking and BMI. Thus, compared with CRP, Lp-PLA₂ has the advantage of being only minimally correlated with other risk factors, which may represent an important advantage for the clinical application of the test.

Strengths and Limitations of the Study
The present study has several strengths. In contrast to WOSCOPS and the WHS, which both used case-control designs, in the present study, a complete cohort, drawn randomly from the general population, has been investigated. Although ARIC also represents a population-based sample, the analysis was done with a case-cohort design. We used only hard coronary end points in our analysis, whereas in the other studies, either revascularization procedures or stroke were included in addition to fatal and nonfatal MI. The considerably longer follow-up of 14 years extends the observations reported from these other studies regarding the time frame within which Lp-PLA₂ might be a useful predictor. The average TC level in the present study, which was lower than in WOSCOPS but higher than in ARIC, finally closes the gap and suggests that Lp-PLA₂ may predict coronary events across all levels of TC.

The present study has several limitations that need to be mentioned. The number of events was rather small, so the power of the study is limited. Also, LDL cholesterol had not been measured for logistical reasons, and therefore, we could not specifically address the question of whether Lp-PLA₂ might be particularly useful as a predictor in those with low LDL cholesterol.

Despite these limitations, our results provide strong evidence for an independent and clinically relevant relationship between elevated concentrations of Lp-PLA₂ and subsequent risk of CHD in apparently healthy middle-aged men from the general population and thus lend further support to the hypothesis that Lp-PLA₂ may be considered as a novel risk marker for CHD. In addition, our results suggest that Lp-PLA₂ and CRP may be complementary in identifying high-risk subjects, and therefore, the combination of both markers may further improve risk assessment. Moreover, because specific inhibitors of Lp-PLA₂ are currently under development, lowering of this enzyme in plasma and/or the vessel wall would represent a novel, promising tool for the treatment of atherosclerosis and thus may open a new avenue to combat this still widespread disease.

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References


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